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Childhood exposure to non-persistent endocrine disrupting chemicals and multi-omic profiles: A panel study

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ABSTRACT

Background: Individuals are exposed to environmental pollutants with endocrine disrupting activity (endocrine disruptors, EDCs) and the early stages of life are particularly susceptible to these exposures. Previous studies have focused on identifying molecular signatures associated with EDCs, but none have used repeated sampling strategy and integrated multiple omics. We aimed to identify multi-omic signatures associated with childhood exposure to non-persistent EDCs.

Methods: We used data from the HELIX Child Panel Study, which included 156 children aged 6 to 11. Children were followed for one week, in two time periods. Twenty-two non-persistent EDCs (10 phthalate, 7 phenol, and 5 organophosphate pesticide metabolites) were measured in two weekly pools of 15 urine samples each. Multi-omic profiles (methylome, serum and urinary metabolome, proteome) were measured in blood and in a pool urine samples. We developed visit-specific Gaussian Graphical Models based on pairwise partial correlations. The visit-specific networks were then merged to identify reproducible associations. Independent biological evidence was systematically sought to confirm some of these associations and assess their potential health implications.

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Results: 950 reproducible associations were found among which 23 were direct associations between EDCs and omics. For 9 of them, we were able to find corroborating evidence from previous literature: DEP - serotonin, OXBE - cg27466129, OXBE - dimethylamine, triclosan - leptin, triclosan - serotonin, MBzP - Neu5AC, MEHP - cg20080548, oh-MiNP - kynurenine, oxo-MiNP − 5-oxoproline. We used these associations to explore possible mechanisms between EDCs and health outcomes, and found links to health outcomes for 3 analytes: serotonin and kynurenine in relation to neuro-behavioural development, and leptin in relation to obesity and insulin resistance.

Conclusions: This multi-omics network analysis at two time points identified biologically relevant molecular signatures related to non-persistent EDC exposure in childhood, suggesting pathways related to neurological and metabolic outcomes.

1. Background

Endocrine disrupting chemicals (EDCs) are a class of chemicals capable of interfering with the endocrine system, and they have been associated with numerous adverse health outcomes (Encarnação et al., 2019). The effects on human health are not limited to the endocrine system, but may include targets in the nervous, cardiovascular, and reproductive systems (Gore et al., 2015). The early stages of life, prenatal, infancy and childhood, which are characterised by rapid development, are of particular interest since they are recognised to be critical periods of vulnerability to environmental exposures (Warembourg et al., 2019; Chen et al., 2022). Children are of special concern in terms of EDC exposure because of their neurodevelopmental and pre-pubertal metabolic state (Braun, 2017). Exposure to neurotoxic agents could disrupt the temporal and regional emergence of critical developmental processes even after birth (i.e., synapse formation and myelination and thyroid hormone metabolism), as demonstrated for childhood phthalate exposure and adverse neurodevelopment (Cho et al., 2010).

These chemicals are ubiquitous and can be found, among others, in pesticides, plastic objects like toys, pharmaceuticals and personal care products, and contaminated food, water and soil (Gore et al., 2015). Broadly, EDCs can be classified into persistent and non-persistent EDCs. Non-persistent EDCs, the focus of this study, are characterised by short half-lives and fast metabolism, and include parabens, phenols, phthalates, and organophosphate pesticides (Schildroth et al., 2022). These classes of non-persistent EDCs are found in many consumer-level products: for instance, phenols can be found in sunscreen lotions due to their ability to filter UV light (Philippat et al., 2015), while phthalates are commonly used as plasticisers (Wang, Zhu, and Kannan, 2019). Exposures among children are especially of concern since they have contact with potentially contaminated soil and dust; they have frequent hand-to-mouth or object-to-mouth activity; they eat, drink and breathe more per body weight compared to adults.

Mechanistic evidence is needed in humans, in particular in childhood, to evaluate the associations between EDCs and health outcomes. The identification of molecular signatures related to EDC exposure may allow the identification of molecular changes in the general population before the clinical onset of disease. Furthermore, considering that *in utero* exposures may be related to later-life diseases (Boekelheide et al., 2012), it is critical to provide mechanistic evidence throughout the life course. In the field of toxicogenomics, omics have proven useful in identifying subtle changes at the gene expression or metabolomic level, with the aim of predicting the toxicity of new compounds (Audouze et al., 2010). These technologies appear to be highly promising also in epidemiological settings, although the number of studies on the associations between EDC exposure and omics is still small. Epigenetics has generally received considerable attention in the field of environmental health (Bollati and Baccarelli, 2010), partially due to the long-term persistence of some epigenetic modifications in response to prenatal exposure to numerous stressors, including nutritional deficiencies and tobacco smoke (Vineis et al., 2017). For instance, genome-wide placenta DNA methylation data were used to investigate the effects of prenatal exposure to phthalates and synthetic phenols, identifying few significant associations with individual CpG sites or differentially methylated regions (Jedynak et al. 2021; 2022). Proteomics, which seems to have received less attention compared to the other platforms, was used to investigate the inflammatory and immunological responses induced by phthalate exposures in a relatively large sample of pregnant women (Ferguson et al., 2015). Metabolomics might represent an ideal platform to study the effects of exposure to EDCs (Bonvallot et al., 2018; Sun et al., 2022). Indeed, several recent studies conducted both in animal models and humans have examined the use of metabolomics data in the context of metabolism disrupting chemicals (Sun et al., 2022). Zhu et al, for instance, researched the association between phthalate metabolites with blood lipid traits both at the metabolite level and as a mixture of chemicals in individuals from three age groups (<18, 18-, and ≥ 60 years) (Zhu et al., 2020b). Zhao et al investigated the use of non-targeted metabolomics to study the effects of exposure to parabens in pregnant women, although the numbers of exposures considered and the sample size are both relatively small (Zhao et al., 2020).

Despite the health and environmental concerns of EDCs, and the increasing availability of exposure and omics data in large cohorts, few studies have investigated the associations between multiple EDCs and molecular biomarkers across multi-omic layers. While the use of multi-omic data is becoming mainstream in medical research to better understand disease aetiology and progression (Hasin, Seldin, and Lusis, 2017), epidemiological research is still focused on single-omic analyses. Considering that different omic data can provide different levels of information, there is ample opportunity for multi-omics studies to better understand how information flows from the initial exposure to the altered biological effects and eventually disease (Gallego-Païls et al., 2021).

In this study, we aimed to identify multi-omic signatures associated with exposure to non-persistent EDCs in school-aged children using repeated sample collection and an integrated network analysis.

2. Material and methods

2.1. Study design and population

We used data from the Child Panel Study, a subpopulation drawn from the HELIX (Human Early-Life Exposome) subcohort, which consists of five existing European longitudinal population-based birth cohorts studies (Vrijheid et al., 2014). The Child Panel consists of 156 school aged children: 28 from BiB (Born in Bradford: United Kingdom), 28 from EDEN (Etude des Determinants pre et postnatals du developpement et de la sante de l'ENfant; France), 40 from INMA Sabadell (INfancia y Medio Ambiente; Spain), 30 from KANC (Kaunus Cohort; Lithuania), and 30 from RHEA (Greece) (Vrijheid et al., 2014). The Child Panel Study had the following inclusion criteria: a) age 6–11 years at the time of the study, with a preference for ages 7–9 years; b) sufficient stored pregnancy blood and urine samples; c) complete address history available; and d) no serious health problems that may affect the performance of the clinical testing or impact the volunteer's safety (e.g., acute respiratory infection).

All participants were followed during a school week between 2014

and 2015, in two time periods (visits A and B) approximately 6 months apart, during which repeated data on exposures, individual behaviours, and phenotypes were collected. The same protocols were used in the two weeks. Details about the panel study design and population can be found in (Casas et al., 2018), while details about sample collection can be found in (Gallego-Paüls et al., 2021). Briefly, each child collected 15 urine samples per week (mean: 14.7 urines per week; SD: 0.7), twice daily (first morning and bedtime voids) in high-quality polypropylene tubes. Non-persistent EDCs are prone to exposure misclassification due to their fast metabolism and high intra-individual variability (Casas et al., 2018). To overcome this limitation, a pool of these 15 samples was used for the EDC measurements. For the omics analyses, the last two urine samples (morning and night samples, when available) collected were used, in order to study omic signatures at the end of each week of exposure data collection. Similarly, blood samples were collected at the end of each week during the clinical examination of the child, ensuring an approximate 3-h fasting time since the last meal.

Among the 156 panel study children, 152 had chemical exposures available, within which 140 and 143, respectively in visits A and B, had all four omic datasets available (see Table 1 for the sample size in each dataset).

All research protocols were approved by the Ethics Committee of each country and informed consent was obtained from all subjects.

2.2. EDCs assessment

We analysed a total of 10 phthalate metabolites originating from 6 distinct phthalate parent compounds, 7 phenols, and 5 non-specific organophosphate pesticide metabolites. Table 2 provides information regarding the EDC nomenclature used in this study, together with compound IDs from the PubChem (Kim et al., 2021) and the Comparative Toxicogenomics (Davis et al., 2021) databases, and their creatinine-adjusted concentrations for each visit. For observations below the limit of detection (LOD), values were imputed by means of quantile regression. Missing values (MEHP: 1; PRPA: 4; BPA: 5; DETP: 7) were imputed using k-nearest neighbours (k = 5) from the VIM R package (Kowarik and Templ, 2016). The concentrations of all the chemicals were log2-transformed to handle their right-skewed distributions. The final dataset consisted of the creatinine-adjusted and log2-transformed levels of 22 chemicals. Further details about the chemical levels and chemical analyses can be found in the Supplementary Methods – EDCs Assessment, and elsewhere (Casas et al., 2018; Haug et al., 2018).

2.3. Omics data

In-depth omics profiling was carried out at the two time points for 140 and 143 children, respectively in visit A and B. Details about laboratory and data processing methods are available in the Supplementary Methods - Processing of omic signatures, quality control and normalization, and elsewhere (Lau et al., 2018; Vives-Usano et al., 2020; Maitre et al., 2021; Gallego-Paüls et al., 2021). Briefly, blood leukocytes DNA methylation screening was based on genome-wide arrays and was assessed with the Infinium HumanMethylation450 beadchip. After pre-processing, quality control and normalisation, the dataset consisted of 386,518 CpG probes. The other molecular layers were screened using targeted or semi-targeted methods. Plasma proteins were measured using three Luminex multiplex assays and included a total of 36 cytokines, apolipoproteins and adipokines. The serum metabolites were measured using a targeted LC-MS/MS metabolomic assay based on the Biocrates AbsoluteIDQ p180 kit, and consisted of 177 metabolites, including amino acids, biogenic amines, acylcarnitines, glycerophospholipids, sphingolipids and sum of hexoses. The urinary metabolites were measured with ^{1}H nuclear magnetic resonance (NMR) spectroscopy, and consisted of 44 metabolites, including amino acids, organic acids, nicotinamides, amines and gut microbial-derived phenols. All measured metabolites were included in the analyses.

Table 1
Study population selection for each visit and each data type in the HELIX panel study.

Data type	Visit A (n)	Visit B (n)
Exposure	152	152
Methylome	149	149
Serum metabolome	155	155
Urinary metabolome	156	154
Proteome	150	155
All data available	140	143

2.4. Bioinformatics and statistical analysis

The statistical and bioinformatics workflows, adopted for each time point separately, are shown in Fig. 1, and are further described below. All statistical analyses were performed using R version 4.0.4 (R Core Team 2021). The code to reproduce the analyses and results of the present study is available in a repository hosted on GitHub.

2.4.1. Dimensionality reduction of the methylation data

The ComBat-corrected (Johnson, Li, and Rabinovic, 2007) methylome was further pre-processed. The minfi R package (Aryee et al., 2014) was used to remove probes containing a SNP at the CpG site or the single nucleotide extension, reducing the dimensionality from 386,518 to 384,955. We further corrected the methylation data using SmartSVA (Chen et al., 2017). Due to the large differences in dimensions of the omics datasets, we chose to reduce the number of CpG sites before computing shrinkage estimates of pairwise partial correlation coefficients by applying a 2-step workflow. First, we filtered out the unreliable CpG probes to keep only “good” and “excellent” probes based on results of a previous study in an independent population (Sugden et al., 2020). Briefly, the reliability was computed using intraclass correlation (ICC) estimates, a commonly used metric to assess reliability in test–retest analyses (Sugden et al., 2020). For the purpose of the present study, we used an ICC cut-off of 0.6. This step reduced the dimensionality from 384,955 to 40,999 CpG sites. Second, we performed visit-specific epigenome-wide association studies (EWAS) to assess associations between EDC levels and DNA methylation. The EWAS was performed with ewaff (Suderman et al., xxxx) and robust linear regression, using the beta values as dependent variables and the EDCs as independent variables (i.e., one model for each combination of beta value and EDC). These models were adjusted for sex of the child, age in years, age- and sex-standardized body mass index (zBMI), cohort, season of visit, and time to last meal. The exposure dataset was standardised using robStandardize from the robustHD R package (Alfons, 2021). Putative outliers in the methylation data were handled using winsorization (default percentile of 0.05). P-values were corrected for false discovery rate (FDR) separately for each chemical class (phthalates, phenols and OP pesticides). The probes with FDR-corrected p-values below 0.2 and reproducible in the two time points were finally selected. This step further reduced the dimensionality from 40,999 to 270 CpG sites. Methylation levels were transformed from beta values (range from 0 to 1) to M-values for downstream analyses (Du et al., 2010).

2.4.2. Pre-processing of the omics data

The urine metabolomics data were adjusted for variable urine sample dilution using probabilistic quotient normalisation (Dieterle et al., 2006). The omic profiles were further adjusted for possible confounders using a 2-stage residual-outcome regression using the lmRob function from the robust R package (Maechler et al., 2021): each omic biomarker was regressed against the possible confounders using robust multivariate regression, and the residuals were extracted and used as adjusted-biomarkers in the downstream analyses. Possible confounders were selected *a priori* and included sex of the child, age in years, zBMI, cohort, and season of visit. Season of visit was considered a potential confounder

Table 2
Non-persistent EDCs analyzed in the HELIX Child Panel. Abbreviations: CTD, Comparative Toxicogenomics Database.

Class	Compound	Abbreviation	PubChem CID	CTD	Urinary concentrations adjusted for creatinine (μg/g). Median (IQR)	
					Visit A	Visit B
OP pesticide metabolites	diethyl phosphate	DEP	654	C034789	3.73 (2.06, 6.48)	3.36 (2.11, 5.37)
	diethyl thiophosphate	DETP	3,683,036	C035638	1.24 (0.22, 2.87)	1.09 (0.14, 2.75)
	dimethyl dithiophosphate	DMDTP	36,158	C040339	0.06 (0.04, 0.39)	0.06 (0.04, 0.28)
	dimethyl phosphate	DMP	13,134	C007477	1.99 (0.23, 5.60)	1.39 (0.21, 5.24)
	dimethyl thiophosphate	DMTP	168,140	C040340	4.96 (3.03, 7.62)	4.54 (2.81, 8.24)
Phenols	bisphenol A	BPA	6623	C006780	4.61 (3.12, 7.25)	3.87 (2.80, 5.46)
	ethyl-paraben	ETPA	8434	C012313	0.88 (0.56, 2.26)	0.74 (0.51, 1.46)
	methyl-paraben	MEPA	7456	C015358	20.70 (5.99, 66.39)	10.24 (4.84, 47.38)
	n-butyl-paraben	BUPA	7184	C038091	0.10 (0.06, 0.19)	0.08 (0.06, 0.14)
	oxybenzone	OXBE	4632	C005290	2.50 (1.04, 6.80)	2.62 (0.95, 7.12)
	propyl-paraben	PRPA	7175	C006068	1.19 (0.30, 9.21)	0.73 (0.23, 4.02)
	triclosan	TRCS	5564	D014260	0.75 (0.45, 1.77)	0.66 (0.42, 1.62)
Phthalate metabolites	mono benzyl phthalate	MBzP	31,736	C103325	6.41 (4.28, 9.75)	4.87 (3.11, 8.97)
	mono-2-ethyl 5-carboxypentyl phthalate	MECPP	148,386	C051450	43.66 (28.97, 69.85)	36.47 (23.99, 56.66)
	mono-2-ethyl-5-hydroxyhexyl phthalate	MEHHP	170,295	C479069	24.49 (16.74, 36.68)	19.93 (14.16, 35.01)
	mono-2-ethyl-5-oxohexyl phthalate	MEOHP	119,096	C080276	14.61 (9.58, 22.65)	11.74 (8.32, 19.91)
	mono-2-ethylhexyl phthalate	MEHP	21,924,291	C016599	3.48 (2.39, 5.80)	3.44 (2.06, 5.28)
	mono-4-methyl-7-hydroxyoctyl phthalate	oh-MiNP	102,401,880	NA	6.16 (3.84, 11.59)	6.25 (3.99, 8.88)
	mono-4-methyl-7-oxooctyl phthalate	oxo-MiNP	102,401,881	NA	3.83 (2.82, 5.81)	3.65 (2.58, 4.74)
	mono-*iso*-butyl phthalate	MiBP	92,272	C575690	52.53 (30.93, 87.55)	46.27 (30.09, 71.38)
	mono-n-butyl phthalate	MnBP	8575	C028577	21.74 (15.66, 36.89)	20.57 (14.68, 32.43)
	monoethyl phthalate	MEP	75,318	C581825	50.61 (30.98, 110.61)	43.40 (26.18, 78.09)

because both exposure levels and omic biomarkers may be influenced by season, for instance due to changes in diet. Serum metabolites were further adjusted for time to last meal. The exposures and omic profiles were then standardised to zero mean and unit variance.

2.4.3. EDCs and omics association analyses

The exposures and the omic profiles were then combined into a single data matrix of dimensions 140 samples × 549 molecules (22 EDCs, 270 blood CpG sites, 177 serum metabolites, 44 urinary metabolites, and 36 plasma proteins) for visit A, and 143 samples × 549 molecules for visit B. These matrices were used to compute shrinkage estimates of pairwise partial correlation coefficients: this approach allows for the integration of heterogeneous data while accounting for indirect relationships between variables (i.e., nodes), thus identifying direct associations between them. We considered as significant the partial correlations with a posterior probability greater than 0.8 (Schäfer and Strimmer, 2005). Briefly, the posterior probability of each potential edge refers to the probability of each edge being nonzero, and it is defined as (1 - local FDR). The matrices of partial correlations corresponding to the two time points were subsequently merged based on: a) correspondence between the nodes forming each edge (i.e., partial correlation), and b) the sign of the partial correlation. The resulting matrix, containing reproducible associations among biomarkers across time points, was depicted as a Gaussian Graphical Model (GGM), with nodes corresponding to features (i.e., EDCs and omic features) and edges corresponding to significant partial correlations. The networks were further described using basic network-level metrics: number of nodes and edges; diameter, defined as the maximum distance between any two nodes; edge density, defined as the ratio between the actual number of edges and the number of possible edges; degree centrality, defined as the number of adjacent edges. The largest connected component of the merged network was further visualised with a heatmap based on the adjacency matrix. To identify structures of highly connected nodes, we performed spectral clustering by means of hierarchical clustering, based on the Ward algorithm, on the first 3 eigenvectors of the symmetric normalised graph Laplacian.

The pairwise partial correlations were computed using the pcor.shrink function and the default arguments from the corpcor R package (Schäfer and Strimmer, 2005). The correlation shrinkage intensity λ, which takes values between 0 and 1 with $\lambda = 0$ resulting in estimates of the empirical correlations, was computed analytically by corpcor (Schäfer and Strimmer, 2005). The p- and q-values were estimated using the network.test.edges function from the GeneNet R package (Schaefer and Opgen-Rhein, 2021). The R packages tidygraph (Pedersen, 2020) and ggraph (Pedersen, 2021) were used to handle and visualise the networks, respectively.

In order to assess the stability of the reproducible associations across time points (i.e., the edges present in the merged network), we performed 100 iterations of bootstrap resampling with the same set of subjects across the two time points.

2.5. Literature search for biological evidence of identified associations

To evaluate the presence of independent supporting biological evidence and health implications of the identified associations between EDCs and omic features, we searched for (1) corroborating literature from both epidemiological and animal studies (i.e., manual literature review) complemented with information from the Comparative Toxicogenomics Database (CTD) (Davis et al., 2021) for the reproducible direct associations between EDCs and omic features; (2) adverse health effects associated with the EDC as retrieved from PubChem (Kim et al., 2021); (3) associated traits and physiological effects for omics previously associated with CpG sites listed in the EWAS Atlas (Li et al., 2019), with metabolites listed in HMDB “Ontology/physiological effects” or proteins in the human protein atlas under “Disease involvement” ; Additionally, genes were mapped to the CpG sites using the EWAS Atlas and the EWAS Catalog (Battram et al., 2021) and protein-coding genes was retrieved from GeneCards (Safran et al., 2021). For the manual literature search (1), we retrieved studies from the PubMed database published before May 2022. When no reference was available for the specific EDCs, we expanded the search by including the respective parent compound.

3. Results

3.1. Study population

Table 3 presents the characteristics of the study population at baseline (visit A) and during the follow-up (visit B). Children included in this study were mostly of European ancestry (92%) and were on average

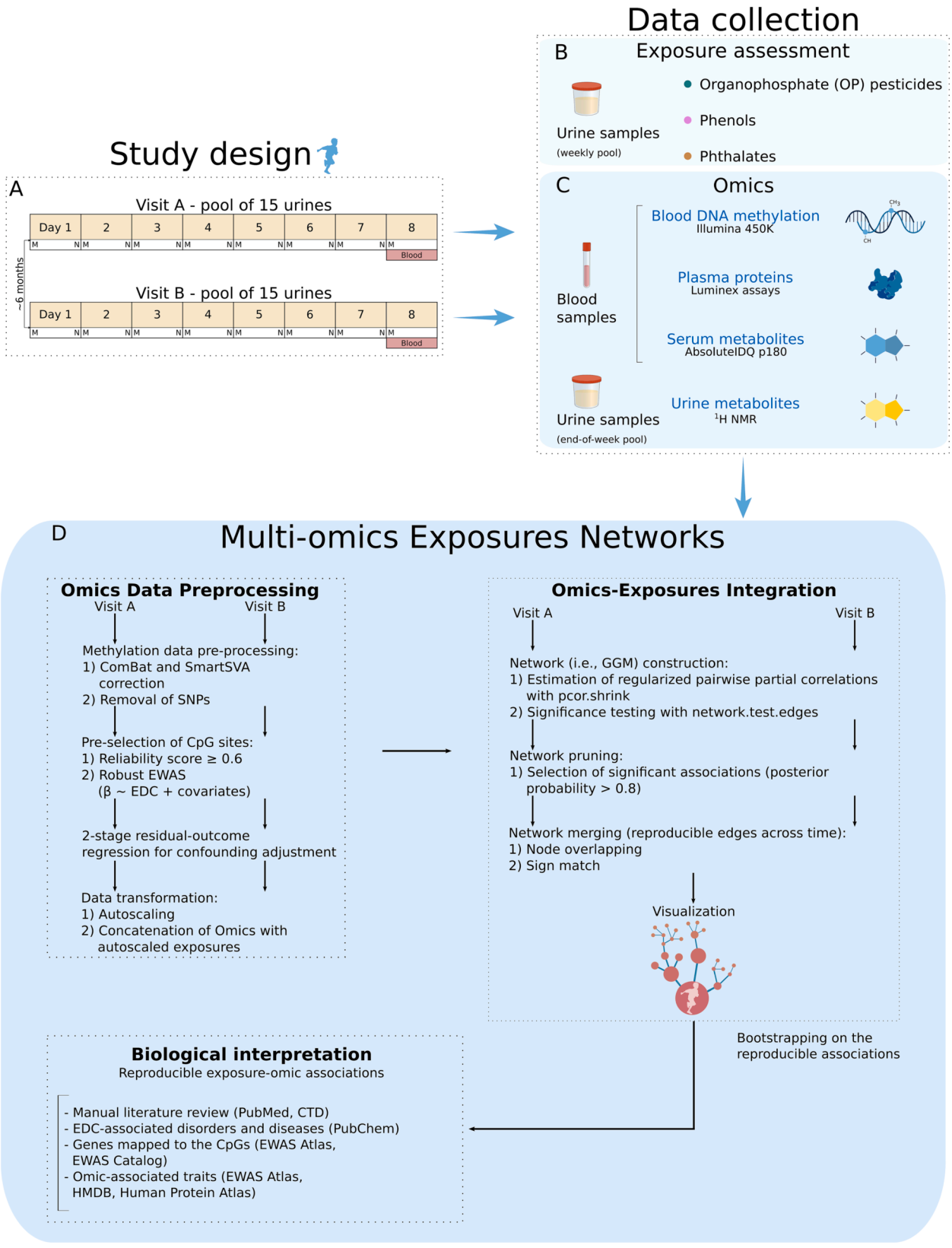

Fig. 1. Overall study design and bioinformatics workflow. A) Study design for the HELIX child panel. B) Exposure assessment: organophosphate pesticides, phenols and phthalates. C) Omics profiling: genome-wide blood DNA methylation and targeted assays for plasma proteins, serum and urine metabolites. D) Statistical and bioinformatics workflow highlighting the main steps, namely: EWAS-based filtering of the CpG sites for dimensionality reduction purposes, 2-stage residual-outcome regression to adjust for confounders, integration of the omics layers and EDCs, estimation of regularized partial correlations, merging of the visit-specific partial correlation networks to identity reproducible associations, bootstrapping to identify the stable associations, and biological interpretation of the reproducible associations.

Table 3
Description of the overall study population, stratified by visit. Abbreviations: zBMI, age standardized z-score for body mass index.

Characteristic	Visit A, N = 140[1]	Visit B, N = 143[1]
Cohort		
BIB	27 (19%)	27 (19%)
EDEN	23 (16%)	23 (16%)
KANC	27 (19%)	26 (18%)
RHEA	29 (21%)	29 (20%)
SAB	34 (24%)	38 (27%)
Sex		
female	60 (43%)	61 (43%)
male	80 (57%)	82 (57%)
Age (years)	6.97 (6.44, 8.85)	7.55 (6.93, 9.62)
Difference between visits (months)	6.05 (4.83, 7.82)	
Ethnicity		
Caucasian	129 (92%)	132 (92%)
Pakistani	10 (7.1%)	10 (7.0%)
Other	1 (0.7%)	1 (0.7%)
zBMI	0.28 (-0.34, 1.09)	0.26 (-0.34, 1.09)
Time to last meal (hours)	3.25 (2.83, 4.09)	2.17 (1.63, 3.17)
Season		
autumn	35 (25%)	38 (27%)
spring	50 (36%)	49 (34%)
summer	20 (14%)	8 (5.6%)
winter	35 (25%)	48 (34%)
Maternal education		
low	15 (11%)	16 (11%)
middle	55 (39%)	55 (38%)
high	66 (47%)	68 (48%)
(Missing)	4 (2.9%)	4 (2.8%)
Tobacco exposure		
no exposure	64 (46%)	63 (44%)
only passive exposure	51 (36%)	53 (37%)
smoker	19 (14%)	20 (14%)
(Missing)	6 (4.3%)	7 (4.9%)

[1]n (%); Median (IQR)

6.97 years old in visit A and 7.55 years old in visit B, corresponding to an average time span between the visits of approximately 6 months. A total of 43% of the children were female. There were no notable differences in characteristics of the study participants between the two visits, with the exception of time to last meal (decreasing from a median value of 3.25 to 2.17 h). Minor differences were also noticeable for season of visit, especially for the number of visits performed during the summer (more in visit A).

3.2. EDCs and multi-omics network analyses

3.2.1. Visit-specific networks

Table 4 presents an overview of the visit-specific edge- and node-level characteristics. No notable differences were observed for the estimate of shrinkage intensity ($\lambda_A = 0.22$ and $\lambda_B = 0.21$) and the diameter (7 in both). While the number of nodes was similar in the two networks (516 and 514, respectively), the network for visit B presented a larger number of edges (4,083 and 4,908 for visits A and B, respectively), which was reflected by a significant increase in the median degree centrality (16 and 21, respectively). The majority of the estimated partial correlations were positive (62% and 60%, for visits A and B respectively) and of small magnitude (interquartile range (IQR) network A: [-0.06, 0.06]; IQR network B: [-0.05, 0.06]). The visit-specific networks also showed remarkably similar distributions of partial correlations and uncorrected p-values (Figure SI[1]).

The five strongest associations in magnitude for each network and their annotated omic features are shown in Table 5. The network for visit A included 323 EDC-omic associations (median partial correlation (mpc): 0.05; minimum: −0.16; maximum: 0.1). The network for visit B, which showed a larger proportion of significant edges (3.26% compared to 2.71% for network A), included 450 EDC-omic associations (mpc: 0.05; minimum: −0.11; maximum: 0.09). The network for visit B further presented a marked increase in the number of edges between CpGs and between CpGs and serum metabolites (Fig. 2).

3.2.2. Merged network: Reproducible associations across visits

To further interpret the associations between EDCs and omics, we focused on the reproducible (i.e., the intersection) edges between the two visits. Table 4 presents an overview of the edge- and node-level characteristics of the merged network. The process of network merging led to the exclusion of a significant number of edges, maintaining 950 edges compared to 4,083 and 4,908 in networks A and B, respectively. Compared to the visit-specific networks, the merged network presented larger diameter (19), smaller median edge density (0.01) and degree centrality (median: 4; IQR: [2, 6]), and a larger proportion of positive edges (81%). All the exposures and proteins, and the majority of the urinary (42 out of 44) and serum metabolites (169 out of 177) were maintained in the merged network.

The process of network merging resulted in layer-specific changes (Fig. 2). The most and least reproducible types of connection were the serum metabolite - serum metabolite (reproducibility score: 65.4%) and protein - urinary metabolites (reproducibility score: 1.2%), respectively. Indeed, the association between monocyte chemoattractant protein 1

Table 4
Edge- and node-level description of the visit-specific and merged networks.

		Visit A Network	Visit B Network		Reproducible Associations (merged network)
Shrinkage intensity		0.22	0.21		
Diameter		7	7		19
Edges (n)		4,083	4,908		950
density		0.03	0.04		0.01
sign[1]	−1	1,547 (38%)	1,956 (40%)		182 (19%)
	1	2,536 (62%)	2,952 (60%)		768 (81%)
partial correlations[1]		0.05 (-0.06, 0.06)	0.05 (-0.05, 0.06)	network A	0.06 (0.05, 0.08)
				network B	0.06 (0.05, 0.08)
q-values[1]		0.024 (0.006, 0.051)	0.023 (0.005, 0.048)	network A	0.009 (0.001, 0.032)
				network B	0.007 (0.000, 0.03)
probability greater than 0.8[1]		4,083 (2.7%)	4,908 (3.3%)		
Nodes (n)		516	514		462
degree[1]		16 (7, 24)	21 (8, 28)		4 (2, 6)
type of node[1]	exposure	22 (4.3%)	22 (4.3%)		22 (4.8%)
	methylome	237 (46%)	238 (46%)		193 (42%)
	serum metabolome	177 (34%)	174 (34%)		169 (37%)
	urinary metabolome	44 (8.5%)	44 (8.6%)		42 (9.1%)
	proteome	36 (7.0%)	36 (7.0%)		36 (7.8%)

[1]n (%); Median (IQR)

Table 5
List of the five strongest EDC-omic associations in magnitude for the visit-specific networks in the children from the HELIX panel study.

Node 1	Layer	Degree	Node 2	Layer	Degree	pcor	qval	Genes
Visit A								
BPA	exposure	24	FGFBasic	proteome	20	−0.156	<0.001	
oxo-MiNP	exposure	21	Trimethylamine oxide	urinary metabolome	30	−0.108	<0.001	
MBzP	exposure	38	BAFF	proteome	18	−0.1	<0.001	
MBzP	exposure	38	MCP1	proteome	29	0.1	<0.001	
MEP	exposure	30	cg00766289	methylome	25	−0.094	<0.001	*TSPAN9*
Visit B								
MEP	exposure	32	cg08158662	methylome	32	−0.109	<0.001	–
BPA	exposure	31	cg22507960	methylome	17	−0.099	<0.001	*TMEM167A;XRCC4*
DETP	exposure	38	cg18628367	methylome	20	−0.096	<0.001	*PTPRN2*
MBzP	exposure	39	Acetate	urinary metabolome	27	0.093	<0.001	
DETP	exposure	38	cg26767081	methylome	32	0.092	<0.001	–

pcor, partial correlation; qval, q-value

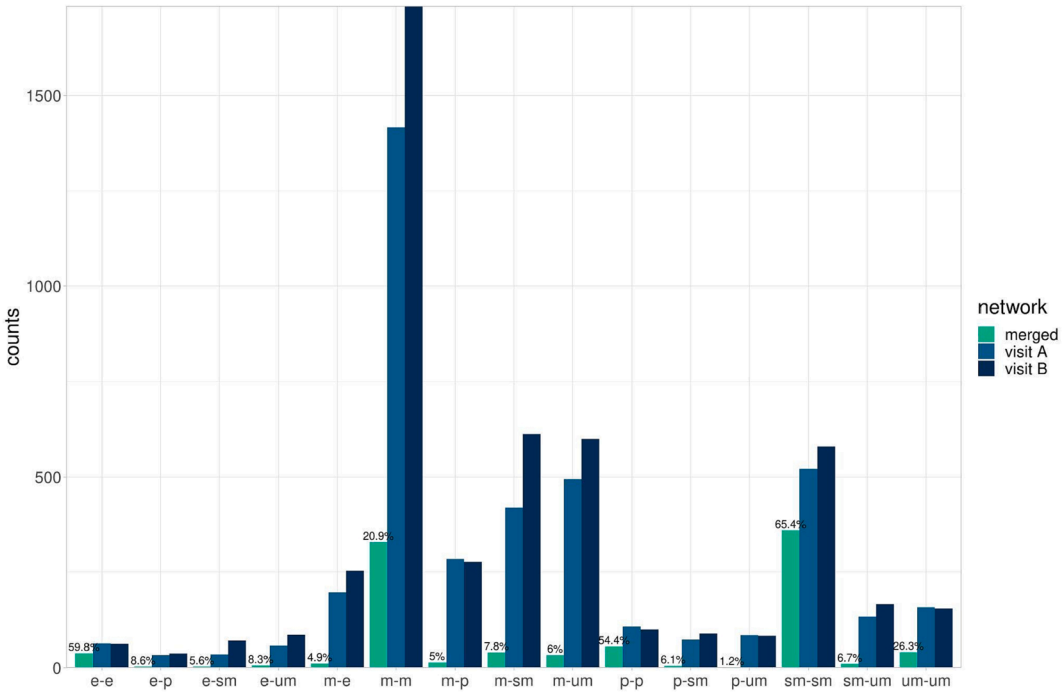

Fig. 2. Grouped barplot showing edge types (e.g., exposure-exposure) for the visit-specific and merged networks. On top of each bar corresponding to the merged network, the reproducibility score, defined as the ratio between the number of edges in the merged network and the average number of edges in the time-specific networks, is reported. Abbreviations: e, exposure (i.e., EDCs); p, plasma proteome; sm, serum metabolome; um, urinary metabolome; m, blood methylome.

(MCP1) and 4-hydroxyphenylacetic acid was the only reproducible association between the proteome and the urinary metabolome. Another type of association exhibiting a low degree of reproducibility was the one between methylation sites and EDCs (reproducibility score: 4.9%). The number of reproducible associations from the EWAS for the two time points, by exposure, is shown in Figure SI 2. Moreover, the majority of the associations found in the merged network were not stable across bootstrapped samples (Figures SI 3 and SI 4). The stable associations between EDCs involved only phthalate metabolites (MEHP, MEHHP, MEOHP, MECPP), and corresponded to the same parental compound (DEHP). Stable associations between CpG sites were investigated using the EWAS Atlas: overall, these associations involved CpGs either annotated to the same gene, or to genes associated to similar traits. cg05566582-cg07514654 were annotated to the same gene (*AC006372.4*); cg06528816-cg11699125 were annotated to *TTC7A* and *ACOT7*, respectively, with both genes associated to similar traits (e.g., asthma and allergic sensitization); cg26647303-cg22580935 were annotated to *MKNK2*; cg05876425-cg18628367 to *PTPRN2*; cg03714522-cg07429146 to *AGAP3*; cg10828456-cg21996245 to *B4GALNT4*; cg26125600-cg20615832 to *PF4V1*. The remaining stable associations were between carnitines, phosphatidylcholines and apolipoproteins. Figures SI 4a and SI 4b show the distribution of the partial correlations for the stable associations across all bootstrapping iterations. Both the graph (Fig. 3) and the heatmap representations (Figure SI 5) reveal the high modularity of the merged network (i.e., higher density of edges within layers). The heatmap of the largest connected component, containing approximately 97% of the nodes, clearly shows the presence of highly connected nodes forming sub-modules within each molecular layer. The largest sub-modules mainly consisted of serum metabolites (phosphatidylcholines, lysophosphatidylcholines, sphingomyelins, and amino acids) or EDCs.

Of particular interest were the reproducible EDC-omic associations (i.e., mixed associations), which represented 2.4% of the total number of edges (950), and which are listed in Table [6] by chemical class and omic layer. Detailed views of the exposure-centred sub-networks with the respective first-degree neighbours, are shown in Fig. 3. These sub-networks highlight some of the reproducible, significant associations between EDCs and endogenous molecules (i.e., proteins, CpG sites and metabolites). The median partial correlation between time points is reported on each edge. Overall, most of these associations included

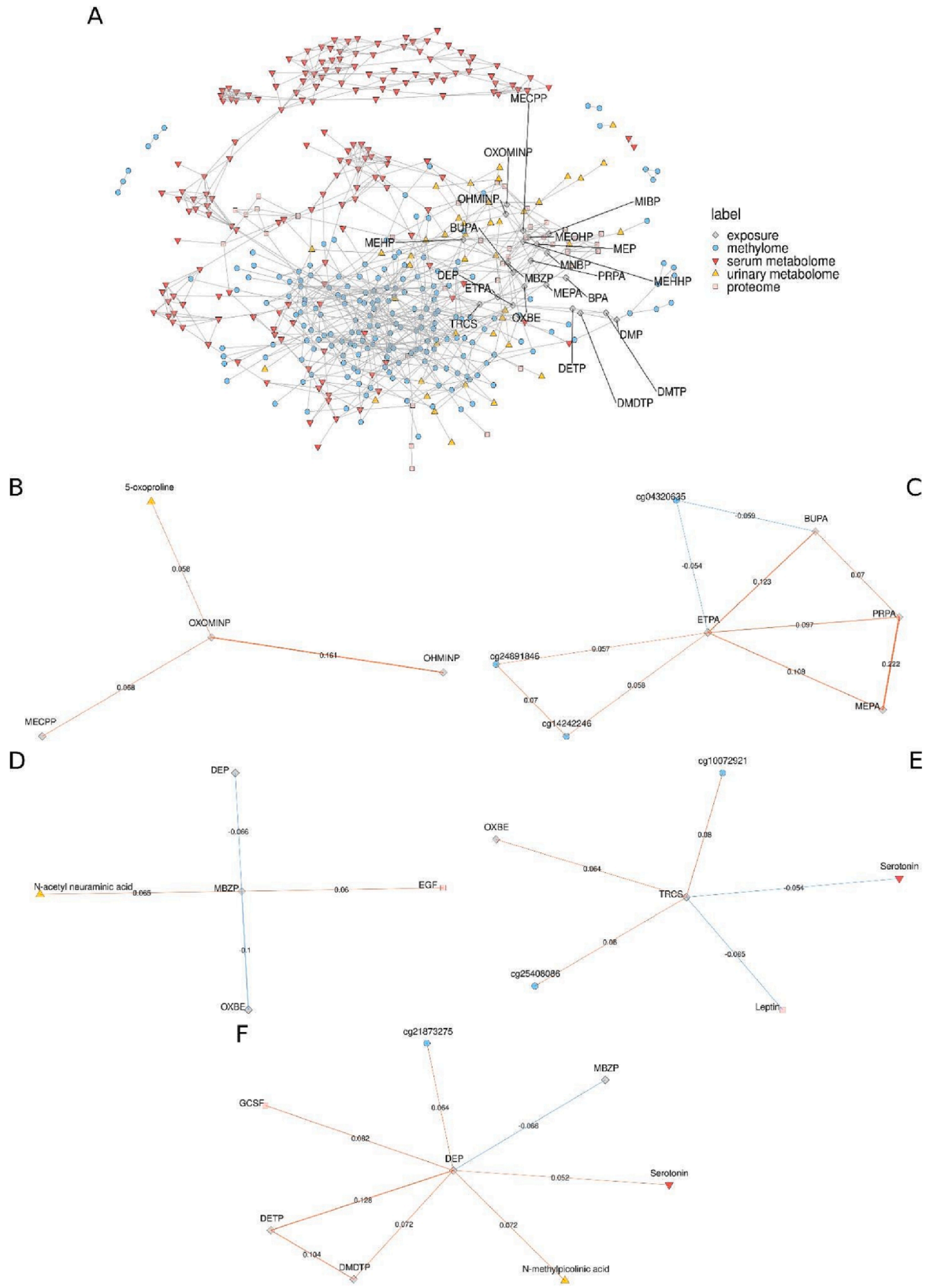

Fig. 3. A) Merged network showing the reproducible edges across time points. The nodes (e.g., metabolites) are color- and shape-coded based on the data layers. To simplify the figure, labels are shown only for the chemicals. The network was rendered with ggraph and the force-directed layout algorithm by Fruchterman and Reingold. B), C), D), E) and F) show the compound-centered networks for the EDCs of interest (i.e., sub-networks containing mixed interactions), with the respective first-degree neighbors. The edges of the compound-centered networks are color-coded based on the sign of the corresponding partial correlation (blue: negative, red: positive), which is displayed on the edges themselves. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

methylation sites (11), followed by urinary metabolites (6), and serum metabolites and proteins (3). Furthermore, the majority of these associations showed similar values of partial correlation across the two visits (median difference: 0.01; minimum difference: <0.01; maximum difference: 0.04). DEP, a non-specific metabolite of multiple OP pesticides, was the only EDC being associated with all four omic layers. The parabens ETPA and BUPA were only associated with CpG sites, one of which in common (cg04320635). The phenol triclosan (TRCS) was associated with omic features belonging to three distinct layers: two CpG sites (cg10072921 and cg25408086), leptin (proteome), and serotonin (serum metabolome). The phthalate metabolites as a group were associated with all four omic layers, although the most representative layer was the urinary metabolome. oh-MiNP and oxo-MiNP, secondary metabolites of the same parental compound (di-isononyl phthalate, DiNP), did not have any molecular feature in common.

3.3. Biological interpretation of reproducible EDC-omic associations

Detailed information from our literature search on the biological interpretation of these associations, by chemical class and omic layer, is provided in Tables 6 and 7.

Among the 23 reproducible associations between EDCs and omic features, we were able to find corroborating independent evidence for 9 of them from previous literature: DEP - serotonin, OXBE - cg27466129 (*KIAA0513*), OXBE - dimethylamine, triclosan - leptin, triclosan - serotonin, MBzP - Neu5AC, MEHP - cg20080548 (*SBSN*), oh-MiNP - kynurenine, oxo-MiNP – 5-oxoproline. These studies are summarized in Table 6 under EDC-omic independent evidence. The CTD only provided evidence for a possible association between triclosan and members of the cyclin family, and between MBzP and members of the EGF family, the rest of the evidence was manually extracted from the literature.

The search of PubChem showed that all the EDCs, with the exception of MEHP and oh-MiNP, had associated health effects. Some of these health effects (e.g., body weight, infertility) were associated with EDCs of different chemical classes. Overall, the integration of our results with the literature search and the EDC-related health effects, suggested related health effects for serum metabolites and a protein: serotonin and kynurenine in relation to neuro-behavioural development effect of DEP, triclosan and oh-MiNP, and leptin in relation to obesity and insulin resistance effect of triclosan. With the exception of cg21873275 and cg25408086, the CpG sites associated with the EDCs in our study all had associated traits in the EWAS Atlas. Some of the adverse health effects associated with exposure to triclosan were also among the traits related to cg10072921.

4. Discussion

We present the first study to assess multi-omics associations with non-persistent EDCs, with a reliable and repeated exposure assessment as well as repeated multi-omic measures, in an European child cohort. The adoption of a multi-omics integrative strategy, that goes beyond the traditional single exposure - single omic association study, revealed short-term associations between non-persistent EDCs (exposures averaged over one week) and multiple omic biomarkers. These results were reproducible in two visits, and some associations were further validated through independent biological evidence. Previous literature provide support for the possible involvement of some of our results in relation to neurological outcomes (DEP - serotonin, triclosan - serotonin, oh-MiNP - kynurenine), and obesity and insulin resistance (triclosan - leptin). Many of the EDCs that we identified as associated with omic markers have, to our knowledge, not been studied in relation to health outcomes in the setting of childhood exposure.

4.1. Biological interpretation

For our discussion regarding the biological interpretation of findings, we focus on the reproducible EDC-omic associations for which independent evidence was available.

Firstly, multiple studies have investigated the possible association between exposure to OP pesticides and the serotoninergic system (Slotkin and Seidler, 2008; Judge et al., 2016): there is now compelling evidence, particularly from experimental models, that exposure to OP pesticides affects serotonin synaptic function and turnover. A recent review further illustrates the possible molecular mechanisms linking exposure to a multitude of chemical stressors, including pesticides, and serotonin turnover (Sarrouilhe, Defamie, and Mesnil, 2021). Serotonin was also among the numerous metabolites affected by exposure to low doses of triclosan in rats (Houten et al., 2016). In the present study, the serum metabolite serotonin was positively associated with DEP, a non-specific metabolite of OP pesticides, and negatively associated with

Table 6
List of the reproducible EDC-omic associations in the children of the HELIX panel study, by chemical class and omic layer. We report the visit-specific partial correlations and q-values.

Chemical class	Chemical	Omic layer	Omic feature	$pcor_A$	$pcor_B$	$qval_A$	$qval_B$
OP pesticide metabolites	DEP	Methylome	cg21873275	0.076	0.053	0.001	0.047
		Proteome	GCSF	0.084	0.08	<0.001	<0.001
		Serum metabolome	Serotonin	0.051	0.053	0.074	0.05
		Urinary metabolome	N-methylpicolinic acid	0.062	0.083	0.017	<0.001
Phenols	ETPA	Methylome	cg04320635	−0.052	−0.056	0.069	0.03
			cg14242246	0.06	0.056	0.021	0.033
			cg24891846	0.063	0.05	0.014	0.066
	BUPA	Methylome	cg03060555	0.069	0.082	0.005	<0.001
			cg04320635	−0.052	−0.066	0.069	0.006
			cg20320656	0.063	0.052	0.015	0.054
	OXBE	Methylome	cg27466129	−0.089	−0.051	<0.001	0.062
		Urinary metabolome	Dimethylamine	−0.071	−0.074	0.003	0.001
	TRCS	Methylome	cg10072921	0.072	0.089	0.003	<0.001
			cg25408086	0.065	0.054	0.01	0.039
		Proteome	Leptin	−0.072	−0.059	0.003	0.02
		Serum metabolome	Serotonin	−0.055	−0.054	0.049	0.042
Phthalate metabolites	MBzP	Proteome	EGF	0.062	0.058	0.016	0.022
		Urinary metabolome	N-acetyl neuraminic acid	0.072	0.057	0.003	0.024
	MEHP	Methylome	cg20080548	−0.057	−0.066	0.037	0.007
		Urinary metabolome	Acetate	−0.063	−0.057	0.013	0.028
	oh-MiNP	Serum metabolome	Kynurenine	−0.051	−0.06	0.074	0.016
	oxo-MiNP	Urinary metabolome	5-oxoproline	0.06	0.056	0.023	0.031
	MiBP	Urinary metabolome	Scyllo-inositol	−0.051	−0.077	0.081	0.001

pcor, partial correlation; qval, q-value

Table 7
Biological interpretation and literature review for the reproducible EDC-omic associations and their potential health implications in children. Associated disorders and diseases to EDCs were retrieved from PubChem ("Associated Disorders and Diseases") [42]. Associated traits and physiological effects for omics were retrieved from the EWAS Atlas for CpGs [43], the Human Metabolomic Database (HMDB) under "Physiological effects" for metabolites, and the Human Protein Atlas under "Disease involvement".

Chemical class	Chemical	Omic layer	Omic feature	Genes	Biological interpretation		
					EDC-omic independent evidence	Associated disorders and diseases (EDCs)	Associated traits and physiological effects (omics)
OP pesticide metabolites	DEP	Methylome	cg21873275	*NBPF25P* [1]	–	Hyperthyroidism [2]; Prenatal exposure delayed effects and abnormal reflex [3]; Respiratory tract diseases [4]	–
		Proteome	GCSF		–		–
		Serum metabolome	Serotonin		Long-term effects of developmental neurotoxicants [5] (†) Link between acute OP exposure and increased activity of 5-HT system [6] (†) Exposure to pesticides linked to changes in the serotonergic system [7] (†)		Nervous system, endocrine (thryoidism) and digestive system disorders
		Urinary metabolome	N-methylpicolinic acid (Homarine)		–		–
Phenols	ETPA	Methylome	cg04320635	*GSE1*;*RP11-118F19.1*	–	Infertility [8]	Smoking; Oestrogen receptor beta status in colorectal cancer
			cg14242246	*C2orf28*; *SLC5A6*	–		Primary Sjögren's Syndrome
			cg24891846	*CACNA1A*	–		Food allergy; Obesity; Adenoma
	BUPA	Methylome	cg03060555	*ZBTB38*	–	Prostatic and testicular diseases [9]; Weight loss [10]	Aging
			cg04320635	*GSE1*;*RP11-118F19.1*	–		Smoking; Oestrogen receptor beta status in colorectal cancer
			cg20320656	*LETMD1*	–		Systemic lupus erythematosus; Atopy
	OXBE	Methylome	cg27466129	*KIAA0513*	OXBE associated with different neurological disorders [11] (†) *KIAA0513* as candidate biomarker for the early diagnosis of AD [12] (‡)	Birth weight [13]; Prenatal exposure delayed effects [14]	Follicular thyroid carcinoma
		Urinary metabolome	Dimethylamine		Fish as a common source of DMA [15] Presence of BP-3 in different species of fish [16]		Renal, and urinary system, digestive system
	TRCS	Methylome	cg10072921	–	–	Hepatotoxicity [17]; Body weight [18]; Fetal growth retardation [19]; Hypersensitivity [20]; Infertility [21]; Inflammation [22]; Insulin resistance [23]	Type 2 diabetes; Infertility
			cg25408086	*CCNT1*	–		–
		Proteome	Leptin		Positive effect of a mixture of phenols and parabens on leptin in black women [24] (‡) No evidence of an association between triclosan and leptin [25] (‡)		Diabetes mellitus and obesity
		Serum metabolome	Serotonin		Possible association between personal care product ingredients and serotonin [26] (†)		Nervous system, endocrine (thryoidism) and digestive system disorders
Phthalate metabolites	MBzP	Proteome	EGF (Epidermal growth factor)		–	Aneuploidy [27]; Birth weight [28]; Body weight [29]; Obesity and insulin resistance [30]	–
		Urinary metabolome	N-acetyl neuraminic (Neu5AC)		Possible association between MBzP and Neu5AC [31] (‡)		Inborn errors of metabolism
	MEHP	Methylome	cg20080548	*SBSN*	PI3K/AKT pathway perturbed by exposure to low doses of MEHP [32] (†) PI3K/AKT pathway associated with *SBSN* [33] (†)	–	Down syndrome
		Urinary metabolome	Acetate		–		Respiratory, nervous and digestive system disorders
	oh-MiNP	Serum metabolome	Kynurenine		Possible mediating effect of quinolic acid on exposure to phthalates and neurological disorders [34] (‡)	–	Nervous system and digestive system disorders

(*continued on next page*)

Table 7 (*continued*)

Chemical class	Chemical	Omic layer	Omic feature	Genes	Biological interpretation		
					EDC-omic independent evidence	Associated disorders and diseases (EDCs)	Associated traits and physiological effects (omics)
	oxo-MiNP	Urinary metabolome	5-oxoproline		Possible association between personal care product ingredients and 5-oxoproline [26] (†)	Total serum testosterone concentrations [35]; Respiratory function (FEV_1%) [36]	Inborn errors of metabolism, digestive disorders
	MiBP	Urinary metabolome	Scyllo-inositol		–	Atherosclerosis [37]; Eczema [38]; Obesity [39]; Premature birth [40]; Prenatal exposure delayed effects [41]	Nervous system disorder

† *In vitro / In Vivo*. ‡ Human study.

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triclosan, a phenol present in several consumer-level products and used as an antibacterial agent. The phthalate metabolite oh-MiNP, a secondary metabolite of DiNP, was negatively associated with kynurenine, a metabolite of L-tryptophan, which is, among others, the sole precursor of serotonin synthesis. Tryptophan metabolism disruption due to exposure to several phthalates was observed in a retrospective cross-sectional study of adult men, although oh-MiNP was not among the measured phthalates (Zhang et al., 2016). Moreover, there is evidence of a possible involvement of quinolinic acid, a metabolite of the kynurenine pathway, for the association between exposure to phthalates and neurological disorders (Nassan et al., 2019). However, there are insufficient studies that investigated health effects of DiNP exposure.

The majority of the phenols were present in the merged network. Parabens, synthetic phenolic compounds, are a class of chemicals ubiquitously used as preservatives in different consumer products. ETPA and BUPA were only associated with methylation sites, of which one, cg04320635, in common. Interestingly, the EWAS Atlas reported obesity as a trait associated with cg24891846, and exposure to ETPA was previously associated with obesity and diabetes mellitus in a large adult population (N = 3782) (Lee et al., 2021). OXBE, present in several cosmetics and widely used as UV radiation absorbent, was negatively associated with dimethylamine. A common source of dimethylamine (DMA) is diet, especially the consumption of fish (Mitchell, Zhang, and Smith, 2008), and there is evidence of the presence of BP-3 in different species of fish (Gago-Ferrero et al., 2012), thus suggesting a possible dietary route of exposure. OXBE was further associated with cg27466129, which was mapped to the gene *KIAA0513*. *KIAA0513* was studied in relation to Alzheimer's disease (AD), being described as a candidate biomarker for the early diagnosis of AD (Zhu et al., 2020a). Interestingly, a previous study investigated the mechanistic response of prenatal exposure to OXBE in neuronal cells (in vivo): based on the altered pathways, the authors concluded that there is evidence of OXBE being associated with the development of different neurological disorders, including AD (Wnuk et al., 2019).

Triclosan was furthermore negatively associated with the protein leptin, and positively associated with two methylation sites. No evidence of an association between triclosan and the peptide hormone leptin was found in a pregnancy cohort (Shapiro et al., 2018), although a more recent study found a positive effect of a mixture of phenols and parabens on leptin in black women (Lee et al., 2022). Traits associated with cg10072921 in the EWAS Atlas included type-2 diabetes and infertility. Considering that exposure to triclosan was previously linked to, among others, body weight (Lankester et al., 2013), insulin resistance (Hua et al., 2017), and infertility (Raut and Angus, 2010), this provides further evidence for a possible involvement of cg10072921 in these pathologies. cg25408086 was mapped to the gene *CCNT1* (cyclin-T1), although the CTD provides only evidence of an interaction between triclosan and *CCND1* (cyclin-D1).

Overall, the phthalate metabolites were associated with multiple omic features involved in different biological pathways. The biological interpretation of these associations was made problematic by the lack of information on adverse health effects from PubChem. MBzP, a metabolite of the plasticiser butyl benzyl phthalate (BBP), was positively associated with the protein EGF and the urinary metabolite N-acetyl neuraminic acid. The CTD provides evidence that MBzP results in an increased expression of EREG, a member of the EGF family. N-acetyl neuraminic, also known as Neu5Ac or NANA, was previously associated in pregnant women with several phthalate metabolites, including MBzP (Zhou et al., 2018). MEHP was negatively associated with cg20080548, which was mapped to the gene *SBSN*. While the molecular details are yet to be understood, there is evidence that this gene has a role, among others, in tumor progression (Tan, Wang, and Liu, 2022). One of the pathways believed to be associated with *SBSN* is the PI3K/AKT pathway (phosphoinositide-3-kinase/Akt pathway) (Tan, Wang, and Liu, 2022), which was reported to be perturbed by exposure to low doses of MEHP in the framework of ovarian cancer progression (Leng et al., 2021). MiBP, a metabolite of di-isobutyl phthalate (DiBP), was negatively associated with *scyllo*-Inositol. Diseases associated with exposure to MiBP include obesity (Lind et al., 2012), and inositols are involved in multiple metabolic pathways, including glucose and lipid metabolism, and insulin signalling (Watkins et al., 2022).

5. Strengths and limitations

Our study has several strengths. The main focus of this study was on non-persistent chemicals, which are prone to exposure misclassification due to their fast metabolism and their high intra-individual variability (Casas et al., 2018). In order to overcome this limitation, we relied on repeated pooled samples of urines across each week (15 samples collected in each visit per child). We investigated the use of biomarkers of exposure and multi-omic data to infer partial correlation networks. Specifically, we focused on four layers: blood DNA methylome, serum and urinary metabolome, and plasma proteome. This approach allows to study interactions not only between exposures and omics, but also between and within each layer, providing an integrative picture of biological pathways at different levels from epigenetic marks to downstream metabolites. This strategy provided useful insights reconstructing known pathways, but mainly allowed to integrate highly correlated markers within omics layers such as for CpGs related to the same genes or serum metabolites (carnitines, phosphatidylcholines and apolipoproteins). Partial correlation and variable shrinkage allowed to account for indirect associations between variables (due to high correlation within layers), thus identifying only direct associations between them, which is of particular interest to identify key omic markers associated with EDCs. Few other studies have used a similar approach to investigate the associations between endogenous molecules and chemical exposures, or for multi-omic data integration (Zierer et al., 2016; Bessonneau et al., 2021). Another commonly used technique for omics data integration, especially in metabolomics-based epidemiology, is Partial Least Squares (PLS): a significant difference between GMMs and PLS-based networks is that the latter do not comprise connections (i.e., edges) between molecules (i.e., nodes) of the same type (e.g., edges between predictors). Finally, we employed different strategies to validate our results, despite the small sample size. First, our study design, with measurements at two time points, allowed us to filter results based on the reproducibility of omic and EDC associations. Indeed, we have previously shown that a large proportion of omic markers, especially inflammatory proteins and urine metabolites, are highly variable within children (Gallego-Paüls et al., 2021), and this was confirmed in our study leading to the exclusion of a majority of the associations. Second, our literature and toxicological database reviews to assess independent corroborating evidence. This should increase our confidence in the obtained results.

Our study also has several limitations. First, the relatively small sample size (approximately 140 children sampled twice) requires further validation in a larger study population. It is known that the physiological and biochemical effects of EDCs can be sex specific, and also that the omic profiles are influenced by sex (Watkins et al., 2016; Lau et al., 2018): the small sample size in this study prevented us from stratifying the analysis by sex, and performing differential network analysis to investigate eventual differences. The small sample size further required us to reduce the dimensionality of the methylome (e.g., reduce the number of CpGs). The strategy adopted (i.e., EWAS) consisted in considering only those CpGs significantly associated with the chemicals. We did not apply any filtering to the other omic layers. This might lead to a sort of "selection bias" at the feature level. While other methods could have been employed (e.g., unsupervised dimensionality reduction), we decided to adopt this strategy because it allowed us to: (1) consider individual CpGs, rather than clusters, which eased the biological interpretation of the reproducible associations, and (2) target the analyses to the main goal of the study, i.e., the direct associations between omics and chemicals. Second, a significance test for the

shrinkage estimates of partial correlation is an open problem (Bernal et al., 2019), and the method employed by (Schäfer and Strimmer, 2005) is known to result in "high" false positive rates (Omranian et al., 2016). However, we tried to attenuate this issue by focusing on the reproducible associations across time points, and by validating the mixed associations with data available in the literature. Third, providing a biological interpretation for the reproducible associations was challenging, particularly due to the lack of a centralised database of associations between metabolites and exposures (e.g., MWAS Atlas), and due to inconsistencies in the naming of both chemicals and metabolites. Moreover, our literature synthesis is likely to suffer from positive results bias, due to positive results being more likely to be published than null results, which might invalidate some of our findings. Finally, similar to many other epidemiological studies, ours might suffer from residual confounding, that hinders any causal conclusion. One possible source of confounding that we did not take into account is diet: further studies would be needed to investigate how dietary factors might modulate the metabolic response to EDC exposures.

6. Conclusion

In this study we employed an integrative method to investigate reproducible associations across time points between non-persistent endocrine disruptor chemicals and multi-omic profiles, consisting of blood methylation, plasma proteomics, and serum and urinary metabolomics data in a child cohort. Exposure assessment consisted of repeated measurements of non-persistent EDCs. We identified several associations that were reproduced in both time points, and that we corroborated with data from a literature review or publicly available databases. Some of these biological signatures point towards the potential biological effects of non-persistent EDCs in relation to the nervous system (OP pesticide metabolites and phenols) and insulin resistance (phenols) in a population of school-aged children. Among the most significant, we found associations between diethyl phosphate and serotonin, triclosan and serotonin, mono–4–methyl–7–hydroxyoctyl phthalate and kynurenine, triclosan and leptin. Follow up studies are needed in prospective study populations with appropriate exposure assessment for non-persistent chemicals to better characterise the health consequences of these associations in children between EDCs and omic variables.

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CRediT authorship contribution statement

Lorenzo Fabbri: Conceptualization, Software, Formal analysis, Writing – original draft, Visualization. **Ronan Garlantézec:** Conceptualization, Writing – review & editing. **Karine Audouze:** Writing – review & editing, Funding acquisition. **Mariona Bustamante:** Investigation, Resources, Data curation, Writing – review & editing. **Ángel Carracedo:** Data curation. **Leda Chatzi:** Investigation, Resources. **Juan Ramón González:** Data curation. **Regina Gražulevičienė:** Investigation, Resources. **Hector Keun:** Data curation, Writing – review & editing. **Chung-Ho E Lau:** Data curation, Writing – review & editing. **Eduard Sabidó:** Data curation. **Alexandros P Siskos:** Data curation, Writing – review & editing. **Rémy Slama:** Investigation, Resources, Data curation. **Cathrine Thomsen:** Investigation, Resources. **John Wright:** Resources, Data curation. **Wen Lun Yuan:** Writing – review & editing. **Maribel Casas:** Data curation, Writing – review & editing, Funding acquisition. **Martine Vrijheid:** Investigation, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Léa Maitre:** Conceptualization, Data curation, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The raw data supporting the current study are available from the corresponding author on request subject to ethical and legislative review.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107856.

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